

# **Standard Operating Procedures for Submission and Testing of Brucellosis Serological Specimens**

**APHIS/Veterinary Services  
Approved Brucellosis Laboratories**



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# Glossary

**Active surveillance:** First Point Testing (FPT), herd testing, diagnostic testing, movement testing, wildlife testing, etc.

**APHIS-approved laboratory:** Laboratory approved by APHIS to perform official brucellosis testing

**Bovine:** Cattle and/or bison

**Cattle Health Center Veterinary Medical Officer (CHVMO):** CHVMOs are located in the VS Hubs. The Cattle Health Center will notify the laboratory of the appropriate CHVMO point of contact.

**First Point Test (FPT):** Testing at first point where livestock may be concentrated, usually a livestock market buying station, etc. First Point Testing is part of active surveillance testing, but specimens collected at first point of concentration, e.g., livestock market, will be recorded on VS Form 4-54 (Brucellosis Test Record – Market Cattle Testing Program).

**Identification device (ID):** Any man-made identification device including, but not limited to, back tag, metal brucellosis ear tag, metal brite (silver) ear tag, radio frequency identification (RFID) ear tag, owner management tags, carcass/house tag (if not removed from the carcass)

**Passive surveillance:** slaughter surveillance

**Non-negative test result:** any test result that is not negative (i.e. suspect or reactor range test result)

**Screening tests:** official brucellosis screening tests are the Rapid Automated Presumptive (RAP) or Buffered Acidified Plate Antigen (BAPA) tests

**Secondary tests:** official brucellosis secondary tests are the Fluorescent Polarization Assay (FPA) and, if FPA test reading is in the suspect range, the Complement Fixation (CF) test may be performed

**Supplemental tests:** brucellosis tests other than official screening and secondary tests used to provide additional information for epidemiological investigations

**Veterinary Services (VS) Form 4-33:** Designated VS Form 4-33 (Brucellosis Test Record) and VS Form 4-33A (Brucellosis Test Record – Continuation Sheet) – OMB approved multi-part forms or Cattle Health Center approved electronic equivalents

**Veterinary Services (VS) Form 4-54:** (Brucellosis Test Record – Market Cattle Testing Program) – OMB approved multi-part forms or Cattle Health Center approved electronic equivalents

**Veterinary Services Form (VS) 10-4:** NVSL Contact Information Update form

**Veterinary Services (VS) Hub:** VS Eastern Hub – Raleigh, NC, VS Western Hub – Ft. Collins, CO

## Acronyms

**ADD:** Assistant District Director

**AIC:** Animal Identification Coordinator or personnel designated by the ADD

**APHIS:** Animal and Plant Health Inspection Service

**BAPA:** Buffered Acidified Plate Antigen test

**BMRT:** NVSL Brucella and Mycobacterium Reagents Team

**CF:** Complement Fixation test

**CHVMO:** Cattle Health Center Veterinary Medical Officer

**DEO:** District Epidemiological Officer

**FPA:** Fluorescent Polarization Assay

**FTP:** First Point Testing

**ID:** Identification device

**NVSL:** National Veterinary Services Laboratories, Ames, Iowa

**RAP:** Rapid Automated Presumptive test

**RFID:** Radio Frequency Identification Device

**SAHO:** State Animal Health Officer (usually the State Veterinarian or State Veterinarian's designee)

**SOP:** Standard Operating Procedure

**UPS:** United Parcel Service

**USPS:** United States Postal Service

**VS:** Veterinary Services

# 1. Purpose

The purpose of this document is to outline the standard operating procedure (SOP) for properly processing, examining, and reporting results for bovine and bison brucellosis passive (slaughter) and active (e.g. first point testing, herd testing, diagnostic testing, movement testing, wildlife testing, etc.) bovine brucellosis surveillance serological submissions. **Bovine brucellosis serologic surveillance includes cattle, bison, and cervids.** Brucellosis surveillance serological submissions include any surveillance as described by the National Bovine Brucellosis Surveillance Plan submitted for official brucellosis testing at APHIS-approved brucellosis laboratories.

## 1.1 Disease

*Brucella abortus*; *Brucella* sp.

## 1.2 Application/Scope

In order to increase efficiency and to facilitate processing, examining, and reporting of brucellosis surveillance serological submissions, this SOP shall be implemented by all APHIS-approved brucellosis laboratories.

# 2. Specimens and Handling

## 2.1 Specimens Required

Bovine brucellosis slaughter surveillance serological specimens for official brucellosis testing consist primarily of specimens from bulls and cows 2 years of age and older. The age of animals for specimens for active brucellosis surveillance serological submissions is specified in brucellosis program regulations and standards.

The Federal or State Animal Identification Coordinator (AIC) shall work with slaughter establishments/facilities and collectors of active bovine brucellosis surveillance specimens to provide collection of good quality specimens, minimum of 6 – 8 milliliters of whole blood, in a Food Safety Inspection Service and laboratory-approved blood collection vial.

Slaughter establishments/facilities and collectors of active bovine brucellosis surveillance specimens will be trained, by the AIC, in appropriate collection procedures. Packaging and shipment of bovine specimens will be arranged in 40 count cell boxes or on rods. Tube numbers are applied with permanent ink on all (or as per agreement between the laboratory and the submitter) collection vials, and man-made identification devices (ID) will be properly recorded on a completed VS Form 4-54 (Brucellosis Test Record – Market Cattle Testing Program) if specimens are collected at a slaughter facility or at First Point Testing (FPT) facility. The ID shall accompany the specimen to the testing laboratory unless an alternative method is described in a signed fee-basis agreement addendum with the slaughter facility for brucellosis specimen collection. ([2.3 Identification Required](#)). If the specimens are from active surveillance other than FPT, they are to be recorded on a VS Form 4-33 (Brucellosis Test Record) and, if needed, VS Form 4-33A (Brucellosis Test Record – Continuation Sheet).

Specimens collected at slaughter facilities designated for national surveillance should be shipped to the APHIS-approved brucellosis laboratory designated in the fee-basis agreement for those facilities.

Specimens collected in a Greater Yellowstone Area State should be shipped to the designated APHIS-approved brucellosis laboratory within that State.

All other specimens for brucellosis testing, e.g. epidemiologic investigation specimens, international shipment specimens, etc., should be shipped to NVSL for brucellosis screening tests if a State/Federal cooperative APHIS-approved brucellosis laboratory is not located within the State of origin of the specimen. **NVSL will charge a user fee for brucellosis testing not required, e.g. specimen testing for international shipment, brucellosis-free herd certification, etc., by the brucellosis program.**

**All brucellosis non-negative screening test specimens, if the initial test laboratory does not have the capability to perform secondary tests, and non-negative secondary test specimens, should be sent to NVSL for confirmatory testing.**

**Specimens referred to NVSL or shipped directly from the field must be accompanied by VS 10-4 NVSL Contact Information Update form in addition to the VS Form 4-54 or VS Form 4-33.**

Specimens should be shipped to the APHIS-approved brucellosis laboratory at the end of each day or at least every other day. However, specimens should not be shipped on a Friday, weekend, or holiday unless the submitter has made appropriate arrangements for receipt directly with the receiving laboratory.

## ***2.2 Retention of Specimens***

Unless forwarded to NVSL, blood serum specimens shall remain at the laboratory until all examinations have been performed, as necessary or requested, in response to non-negative screening examinations.

If the **entire** collection submitted from a slaughter facility for a kill/collection date or the **entire** collection for an active brucellosis surveillance collection location/date has negative screening test results, the specimen collection will then be properly disposed of using laboratory procedures.

If a specimen has a screening test positive result, the 9 specimens before and the 9 specimens after the positive specimen shall be retained by the laboratory until the laboratory supervisor, the District Epidemiological Officer (DEO), and State Animal Health Official (SAHO) or their designee of the State of origin of the animal from which the specimen originated, and a Cattle Health Center Veterinary Medical Officer (CHVMO) collaboratively determine that the specimens may be properly disposed of using laboratory procedures.

**Any specimen yielding a non-negative test interpretation on a brucellosis secondary test shall remain at the laboratory and be preserved within the laboratory's archives, unless it is sent to NVSL. Serum from non-negative specimens, as determined by the secondary examination, shall be stored in a laboratory freezer (-20 degrees C) and archived for no less than one year.**

### **2.3 Identification Required**

**All** physical identification, including but not limited to: man-made IDs, i.e. back tag, metal brucellosis ear tag, metal brite (silver) ear tag, radio frequency identification (RFID) ear tag or device, owner management tags, carcass/house tag, sale tag, etc., shall be removed from the animal, recorded, and correlated with the blood specimen. Place all of the animal's IDs into an individual 3-hole plastic ID bag correlated to the specimen tube number. **If the carcass tag is not removed from the carcass, the information should be recorded in such a manner that it can be correlated to the blood specimen.**

The Assistant District Director (ADD) may allow the use of any alternate method for handling, recording, and retaining the IDs (i.e. by whom, how, and for how long the IDs must be handled and retained) if the alternate method would provide a ready means of identifying and correlating the blood specimen to a specific carcass with its corresponding ID. The alternative method shall be described in a signed fee-basis agreement addendum with the slaughter facility for brucellosis specimen collection. The AIC shall be responsible to assure compliance with the provisions described in the alternative method.

The fee-basis agreement addendum shall describe when the IDs from animals with negative test results may be disposed, who will notify the slaughter facility regarding when the ID collection may be disposed, and the frequency of the ID disposal notifications. **The IDs for negative specimens must be retained at least until all necessary test results are reported to the DEO of the State of origin of the specimen.**

If the specimens are active brucellosis surveillance serological submissions, **all** IDs shall be recorded on the appropriate VS form: if the collection is FPT, VS Form 4-54; for other active brucellosis surveillance serological submissions use VS Form 4-33 and if needed, VS Form 4-33A.

### **2.4 Retention of Identification Collected with and Accompanying Slaughter Blood Specimens**

IDs that have been collected and correlated with the blood specimens will remain at the laboratory or slaughter facility, as per the fee-basis agreement between the ADD and the slaughter facility, until all secondary and supplemental testing necessary or requested has been conducted on the specimen.

If the **entire** collection submitted from a slaughter facility for a kill/collection date has negative screening test results, the IDs accompanying the blood specimens will be properly disposed of using laboratory procedures after test results are reported to the DEO of the State of origin of the specimens.

If the **entire** collection submitted from a slaughter facility for a kill/collection date has negative screening test results and the IDs were retained at the slaughter facility, the ID collection will then be properly disposed of using procedures described in the signed fee-basis agreement with the slaughter facility. The fee-basis agreement addendum with the slaughter surveillance facility will describe when IDs may be disposed. The addendum will include who will notify the facility and the frequency of the ID disposal notifications.



## **2.5 Retrieval, Recording and Retention of Animal Identification for Brucellosis Test Non-negative Specimens**

Any specimen yielding a non-negative screening test result, regardless of any additional secondary or supplemental test results, shall have any and all IDs correlated with that non-negative specimen and the IDs of the nine animals before and the nine animals after the non-negative animal retrieved, recorded, and retained. ([7.10 Retrieval of ID for Non-negative Specimens](#), [7.11 Recording ID for Non-negative Specimens on a New VS Form 4-54 or VS Form 4-33](#), and [7.12 Retention of Records](#)).

If the ID did not accompany the blood specimen to the laboratory, the AIC of the State of origin of the specimen shall retrieve the ID of the non-negative animal and the ID of the nine animals before and the nine animals after the non-negative animal and send those IDs to the DEO of the State of origin for that animal. A record of the identification will be made and a copy sent by the AIC to the laboratory to be kept with the specimen records that are housed in the laboratory ([7.12 Retention of Records](#)). If the ID does not accompany the blood specimen, it shall be the responsibility of the AIC to assure that ID is retained and can be correlated to specimens as described in fee-basis agreement addendum.

IDs from non-negative specimens shall be retained until the DEO, in collaboration with a CHVMO, determines retention is no longer necessary.

## **3. Examinations**

### **3.1 Assessment**

All bovine brucellosis surveillance submissions received at the APHIS-approved brucellosis laboratory will have overall general quality assessed ([7.5 Assessment](#)) and recorded after processing of the specimens has been conducted. ([7.4 Processing](#))

Bovine brucellosis surveillance specimen submissions that display an untestable quality, e.g. hemolyzed specimens, specimen degradation, specimens with insufficient quantity of serum, etc., shall have documentation ([6.2 Reporting of Test Specimen Quality](#)) created depicting the condition of the specimens. If the laboratory determines that a specimen is untestable due to extremely poor quality, the specimen shall be disposed of using appropriate laboratory procedures ([7.5.3 Specimens of Extremely Poor Quality](#)).

**Submission of high quality brucellosis test specimens is vital to obtaining accurate test results and statistically valid data for brucellosis surveillance analysis and other brucellosis testing.**

### **3.2 Examination**

After the assessment, the specimens shall be examined for detection of antibodies to *Brucella abortus*, *sp.*, using serological examinations in series format.

Specimens shall be prepared for examination as described in [7.6 Preparation](#).

Series testing format: initial serological screening examination using the Rapid Automated Presumptive (RAP) ([7.7.1 Rapid Automated Presumptive \(RAP\) Test](#)) or

Buffered Acidified Plate Antigen (BAPA) ([7.7.2 Buffered Acidified Plate Antigen \(BAPA\) Test](#)), followed by the Fluorescent Polarization Assay (FPA) test ([7.8.1 Fluorescent Polarization Assay \(FPA\)](#)) as the secondary examination if the RAP or BAPA test result is positive. If the FPA test yields a non-negative test interpretation (suspect or positive), the specimen should then be tested using the Complement Fixation test (CF) ([7.9.1 Complement-Fixation Test \(CF\)](#)) as an additional secondary examination. If the FPA and/or CF test is not available at the initial laboratory or the initial laboratory is not approved to perform the FPA or CF test, NVSL shall perform the FPA and CF tests when it receives the specimen for confirmation of screening and secondary test interpretations. The DEO of the State of origin of the specimen shall provide a courier (FedEx or UPS) account number or USPS postal frank to cover charges for directing such requests to NVSL.

All screening and secondary test results will be recorded on the appropriate approved VS Form ([6.1.1 Codes/Test Results for Recording on Forms](#)).

The laboratory shall report all negative screening examination test results to the DEO of the State of origin of the specimen ([6.3.1 Reporting Negative Screening Test Results](#)). All non-negative secondary examination test results shall be reported to the DEO in the State of origin of the specimen ([6.3.2 Reporting of Non-Negative Specimens/Collections](#)).

Secondary examination non-negative specimens shall remain archived at the testing laboratory for no less than one year ([2.2 Retention of Specimens](#)).

The laboratory supervisor, a CHVMO, the DEO of the State of origin of the specimen, or the DEO of the State of origin of the animal may request an additional secondary test or tests be performed if the specimen is of poor quality, a non-negative secondary examination result is obtained, or for epidemiological investigation reasons. If the examination is available at the initial APHIS-approved brucellosis testing laboratory, the request for additional testing shall be made to the laboratory supervisor of that laboratory. If additional secondary and/or supplemental examinations are necessary or requested and the requested additional examination is not available at the original testing laboratory, an official request will be submitted by the requestor to the laboratory supervisor and the specimens will be sent from the laboratory to NVSL per the request of the laboratory's supervisor, the DEO, or the CHVMO. The requestor shall provide a FedEx or UPS account number or USPS postal frank to the laboratory to cover charges for directing such requests to NVSL. In addition to the new VS 4-54 or VS 4-33 ([6.3.2 Reporting of Non-Negative Specimens/Collections](#)), the submitting lab must enter the State of origin of the specimen on the NVSL Contact Information Update VS 10-4 submission form and the animal's State of origin, if known, to ensure appropriate and timely report distribution.

If supplemental examinations are performed, the examination results shall be reported by the testing laboratory to the appropriate VS hub brucellosis CHVMO and the DEO of the State of origin of the animal.

NVSL will report test results through normal APHIS reporting channels to the submitter and to the email groups of the submitter, State of specimen origin, State of animal origin, and State of animal owner (provided this information is available on the submission form.)

Specimens sent to NVSL will routinely be tested and reported within 4 business days of receipt. Test results may be reported sooner depending on tests required and day of the week received. Poor quality specimens, holidays, or invalid tests may occasionally cause a delay. Prior to sending submissions with greater than 25 specimens or to request urgent testing, contact the Serology Section at (515) 337-7563. Specimens should not be shipped on a Friday, weekend, or holiday unless the submitter has made appropriate arrangements for receipt directly with NVSL.

## 4. Calibration Procedures

### 4.1 Calibration

All instruments used in support of examinations conducted in APHIS-approved laboratories will undergo routine maintenance and instrument qualification procedures as recommended by the instrument manufacturer's Operator's Manual. NVSL will service RAP and FPA test equipment owned by APHIS on a biennial basis. Other equipment shall be calibrated, at the laboratory's expense, on a biennial basis by a source that follows the International Organization for Standardization (ISO) accredited requirements. Certificates of Acceptance and Conformity will be kept on file. Instruments shall also be calibrated by personnel within the laboratory to manufacturer's specifications in the event that performance of the instrument has been affected.

## 5. Materials

### 5.1 Reagents

The NVSL Brucella and Mycobacterium Reagents Team (BMRT), Ames, IA, will provide reagents for the BAPA and RAP as well as the reagents for secondary examinations to the APHIS-approved national brucellosis surveillance laboratories. Other APHIS-approved laboratories may purchase test reagents from the NVSL BMRT.

#### 5.1.1 Buffered Acidified Plate Antigen (BAPA) Examination

*Brucella abortus* complement fixation positive control serum (NVSL reagent 12-series control sera)

*Brucella abortus* complement fixation negative control serum (NVSL reagent 12-N)

Buffered *Brucella* sp. plate antigen (BAPA test) (NVSL reagent 2)

#### 5.1.2 Rapid Automated Presumptive (RAP) Examination

Buffered *Brucella abortus* (BBA)/RAP test antigen (NVSL reagent 10-S)

*Brucella abortus* RAP positive control antisera (NVSL reagent 612-RP)

*Brucella abortus* RAP negative control antisera (NVSL reagent 612-RN)

#### 5.1.3 Fluorescence Polarization Assay (FPA)

*Brucella abortus* Antibody Test Kit Fluorescence Polarization Assay may be purchased from:

Ellie, LLC

10437 Innovation Drive

Wauwatosa, WI 53226

Phone/Fax (800) 556-6953

Email: [hello@ellielab.com](mailto:hello@ellielab.com)

Customer Service: Mr. Marcus Loudermilk ([marcus@ellielab.com](mailto:marcus@ellielab.com))  
Technical Support: Dr. Miladin Kostovic ([miladin@ellielab.com](mailto:miladin@ellielab.com))

### 5.1.4 Complement-Fixation Test

*Brucella abortus* complement fixation negative control serum (NVSL reagent 12-N)  
*Brucella abortus* complement fixation low positive control serum (NVSL reagent 12-L)  
*Brucella abortus* complement fixation positive control serum (NVSL reagent 12-series control sera)  
*Brucella abortus* complement fixation high positive control serum (NVSL reagent 12-H)  
Preserved sheep cells  
Hemolysin (rabbit antiserum against sheep erythrocytes)  
Complement (guinea pig)  
Antigen B. abortus (NVSL reagent 3)  
Cyanmethemoglobin reagents and standards  
Sodium barbital and barbital or commercial Veronal Buffered Diluent (VBD)

### 5.2 Supplies

NVSL will provide, upon request, a list of supplies necessary for performing approved brucellosis tests.

### 5.3 Equipment

NVSL will provide, upon request, a list of equipment necessary for performing approved brucellosis tests.

## 6. Reporting

### 6.1 Screening and Secondary Examination Results

Results shall be recorded on VS Form 4-54 or VS Form 4-33 in the column for the specific examination that was performed using the codes as per 6.1.1 Codes/Test Results for Recording on Forms.

#### 6.1.1 Codes/Test Results for Recording on Forms

BAPA Examination	
Code/Test Result Recorded on Forms	Result
N	Negative
P	Positive

RAP Examination	
Code/Test Result Recorded on Forms	Result
N	Negative
P	Positive

FPA Examination	
Delta mP Result Recorded on Forms	Test Interpretation (Do Not Record on Forms Except for Animals to be Exported Internationally)
Delta mP value	Negative (delta mP 1-10)
Delta mP value	Suspect (delta mP 11-20)
Delta mP value	Suspect (delta mP 11-40) <b>Provided the CF test results are negative</b>
Delta mP value	Positive (delta mP $\geq$ 21) <b>Provided the CF test results are positive, anti-complementary, or not available</b>
Delta mP value	Positive (delta mP $\geq$ 41) <b>Regardless of CF test results</b>

**Note:** FPA test results will be recorded as the delta mP reading (reading minus the negative control reading) of the specimen. For example, if a specimen had a reading of 125 and the negative control reading was 85, then the delta mP result will be recorded on the VS Form 4-54 or VS Form 4-33 as "40".

Complement Fixation Test (CF)	
Test Result Recorded on Forms	Test Interpretation (Do Not Record on Forms)
1+ 1:10 or lower	Negative
2+ 1:10 through 1+ 1:20	Suspect
2+ 1:20 or higher	Reactor

**Note 1:** CF dilution results, e.g. 1+ 1:10, 2+ 1:20, etc. shall be recorded on the VS Form 4-54 or VS Form 4-33.

**Note 2:** Anticomplementary test result should be reported as AC. A titer should not be reported if CF test is AC. The following note may be attached to the specimen test form (VS Form 4-54 or VS Form 4-33).

**Note 3:** AC = anticomplementary result = indeterminate result. The factors causing an anticomplementary result are not known but the test may be successful with a new sample from the animal. Specimens should have clear serum, without evidence of hemolysis, for complement fixation or other serologic tests. {USDA APHIS NVSL SOP on *Brucella abortus* CF (SOP-SERO-0015)}

## 6.2 Reporting of Test Specimen Quality

Submission of high quality brucellosis test specimens is vital to obtaining accurate test results and statistically valid data for brucellosis surveillance analysis and other brucellosis testing.

### 6.2.1 Specimen Quality Abbreviations

Specimen quality abbreviations should be recorded and specific reasons why a specimen was deemed unfit/invalid should be noted.

Specimen Quality Abbreviations		
Abbreviation	Term	Description
C	Contaminated	Specimen contains cloudy, “creamy,” translucent to opaque serum
QNS	Quality Not Sufficient	Not enough serum present to perform examinations; includes specimens that may have leaked, caps not properly sealed, etc.
M	Missing	Specimen tube number recorded on the test form, but the specimen tube was not present when specimen shipment was received at the laboratory
NID	No Identification	No identification was included with specimen
H	Hemolysis	Specimen displays extreme hemolysis: thick, viscous liquid with no testable serum
INV	Invalid	Specimen that may appear “good” yet produces an invalid result
GEL	Gel	Specimen displays gelatinous qualities: solid, “gelled” specimen with little to no testable serum
B	Broken	Broken collection tube indicative of a shipping problem
LA	Laboratory Accident	Laboratory accident; mishap in the laboratory prevented specimen from being examined

Each APHIS-approved brucellosis laboratory shall provide, by the 10<sup>th</sup> day of each month, to the designated CHVMO a monthly Brucellosis Test Specimen Quality Submission Report regarding brucellosis surveillance specimens submitted to the laboratory to facilitate monitoring and auditing the quality of test specimens. The total number of unsuitable for testing, invalid test result, and missing specimens for any month should be less than five percent of the total number of specimens collected and submitted by a submitter. Fee-basis agreement submitters may not be paid for specimens of untestable quality if the untestable quality is due to unsatisfactory collection, handling, or submission.

The designated CHVMO shall monitor and audit the quality of brucellosis test specimens submitted to APHIS-approved brucellosis test laboratories to assure high quality, testable specimens are being submitted for testing. The CHVMO shall notify the designated District personnel for the State from which the specimens were submitted if the number of unsuitable for testing, invalid test result, and missing specimens exceeds five percent and are thus not to be authorized for payment as per the fee-basis agreement.

If a significant number (**five percent or more of the total number of specimens submitted from a submitter**) of specimens submitted for brucellosis testing are unsuitable for testing, the designated CHVMO shall forward a copy of the report to the designated District personnel for the State of origin of the specimens. At the

direction of the ADD, the DEO or AIC of the State of origin of the untestable specimens shall investigate the source (slaughter facility, FPT facility, or collecting veterinarian) of the unsuitable specimens to determine the reason(s) for the unsuitable specimens and work with the submitting person or facility to improve the quality of the specimens being submitted.

A Brucellosis Test Specimen Quality Submission Report template is provided on the [Brucellosis Disease Information](#) page.

### **6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results**

#### **6.3.1 Reporting Negative Screening Test Results**

The laboratory shall, **within five work days** of completion of the brucellosis test(s), **report all negative** brucellosis screening test results to the DEO of the State from which the blood specimens were submitted.

Bovine brucellosis surveillance specimens that have a negative test interpretation shall have test results recorded on the original VS Form 4-54 or VS Form 4-33.

The VS forms shall be electronically submitted and/or mailed by the laboratory to the DEO of the State of origin of the specimens.

#### **6.3.2 Reporting of Non-Negative Specimens/Collections**

**Reporting of non-negative brucellosis test results shall be done via telephone and either electronic internet communication (e-mail) or FAX.**

The laboratory shall, **within one work day or sooner** of the completion of the brucellosis FPA secondary examinations, report all **non-negative** FPA test results to the DEO, the ADD of the State of origin of the blood specimens, and to the designated CHVMOs in both VS Hubs (Eastern and Western). The laboratory supervisor shall identify such specimens with a “withheld” (W) test interpretation notation.

If the State of origin of the specimen is not the same as the State of origin of the animal, the DEO of the State of origin of the specimen will, **within one work day or sooner** of receiving the test results, report the findings to the DEO of State of origin of the animal.

The DEO or ADD in the State of origin of the specimen or DEO or ADD in the State of origin of the animal or a CHVMO may request additional brucellosis testing of the non-negative brucellosis test specimens prior to classification of the animal's brucellosis status.

A minimum of brucellosis screening and, if performed, secondary examination test results for the 9 specimens prior to the non-negative specimen and the 9 specimens following shall also be reported. **Screening examination and secondary examination of non-negative specimens shall have IDs ([2.3 Identification Required](#)) retrieved and recorded on a new, separate VS Form 4-54 or VS Form 4-33 and all test results shall be recorded on the new VS Form 4-54 or VS Form 4-33 in the appropriate column for each examination. A copy of the new VS**



**Form 4-54 or VS Form 4-33 and VS Form 10-4 shall accompany the original forms and be sent (initially electronically, followed by mailed hard copy), to the DEO of the State of origin of the specimen. See [2.2 Retention of Specimens](#) and [7.11.1 Recording of ID for Non-Negative Specimens](#) for additional information regarding retention of specimens and IDs.**

**If confirmation of non-negative brucellosis test results is performed at a reference laboratory** (e.g. NVSL), the reference laboratory shall report, within one work day or sooner of completion of the brucellosis test(s), all brucellosis test(s) results to the DEO and the ADD of the State of origin blood specimen and to a brucellosis CHVMO in both VS Hubs .

## **7. Procedures**

### ***7.1 Receiving Submissions via United States Postal Service (USPS)***

Bovine serological specimens submitted to the APHIS-approved laboratory will arrive either by mail courier or will be directed to the box designated for the laboratory at the Post Office. Specimens should arrive in insulated coolers, typically insulated hard plastic coolers (e.g. Coleman). Specimens directed to the P.O. Box shall be retrieved by a designated technician at the beginning of each work shift. Upon returning to the laboratory, the technician shall conduct a count and record the total number of coolers received.

### ***7.2 Receiving Submissions via a Mail Courier, (FedEx, UPS, etc.)***

Bovine serological specimens arriving at the APHIS-approved laboratory via a mail or other courier will be received by the designated technician at the laboratory shipping/receiving area. Specimens should arrive in insulated coolers, typically insulated hard plastic coolers (e.g. Coleman). The technician shall count and record the total number of coolers that were delivered, verify the number with the courier, and sign for receipt of this volume.

### ***7.3 Grouping***

A technician shall sort and group each of the vessels containing bovine serological specimens by collection location or plant/slaughtering facility as well as by kill date if specimens are from a plant/slaughter facility.

### ***7.4 Processing***

Laboratory personnel shall retrieve paperwork from each collection and sort “bundles” (“bundle” describes ID bags containing specimens and identification bound by rod, string, etc.; each collection may contain several “bundles” in multiple coolers) in ascending order as indicated by the VS Form 4-54 or VS Form 4-33 that is to accompany each collection. Additional forms that are included with collections, (i.e. kill sheets, lot logs, etc.) shall accompany the VS Form 4-54 or VS Form 4-33 as well as the specimens throughout the remainder of the process.

Bovine whole blood specimens may arrive in 40 cell-count cardboard boxes in ascending order. If specimens do not arrive in 40 cell-count cardboard boxes, the specimens should be removed from the 3-hole plastic ID bags and placed in sequential order into 40 count boxes or into blood tube racks from left to right, front to back.



The boxes shall be placed in sequential order on carts or tables found within the processing area of the laboratory. Each tube (unless an alternative agreement between the laboratory and the submitter is described in the fee-basis agreement addendum) must be numbered in sequential order. Specimens are centrifuged and examined for specimen quality. After specimens are centrifuged, they are placed in order on a cart and placed in a refrigerator until they are racked for testing.

“Bundles” of ID bags containing identification shall be placed in clear plastic bags and have collection location or slaughter facility/plant name, kill/collection date, received date, and total number of specimens clearly labeled on the outside of the bag and retained in the laboratory until testing of specimens is completed.

## **7.5 Assessment**

Bovine slaughter surveillance submissions and active (e.g. FPT, farm/ranch testing, epidemiologic testing, etc.) surveillance submissions will have overall general quality assessed and recorded (assessment recorded on a worksheet that accompanies VS Form 4-54 or VS Form 4-33 after processing of the specimens has been completed).

### **7.5.1 Specimens of Overall Good Quality**

Specimens of good quality (collections containing ample amounts of serum per specimen and displaying few to no signs of hemolysis, gel, or other degradation) will undergo the RAP ([7.7.1 Rapid Automated Presumptive \(RAP\) Test](#)) screening examination.

### **7.5.2 Specimens of Overall Poor Quality**

In the event that a collection displays an overall poor quality (i.e. collections containing limited amounts of serum, displaying multiple cases of hemolysis, gel, or other degradation), the entire collection will, at the discretion of the of the laboratory supervisor, be tested using the BAPA as the screening assay test (an alternative screening examination) to reduce any complications associated with poor specimen quality. Documentation will be created depicting the condition of the specimens ([6.2 Reporting of Test Specimen Quality](#)) and reported as described in [6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results](#). The documentation should also contain photographs, if possible, and a brief, written description of the specimen(s) indicating the quality and specifics that may have caused the poor conditions, (i.e. no ice packs, delayed shipping, etc.). This documentation will be provided to the laboratory supervisor to be forwarded to the DEO of the State of origin of the specimen.

### **7.5.3 Specimens of Extremely Poor Quality**

In the event of extremely (i.e. specimens displaying extreme hemolysis, extreme specimen degradation, contamination, containing little to no testable serum, and/or other degradation) poor specimen quality resulting in specimens yielding invalid RAP and/or BAPA test results, the specimens will be tested using the FPA secondary examination as screening test. Documentation will be created depicting the condition of the specimens ([6.2 Reporting of Test Specimen Quality](#)) and reported as described in [6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results](#). The documentation should also contain photographs, if possible, and a brief, written description of the specimen(s) indicating the quality and specifics that may have caused the poor conditions, (i.e. no ice packs, delayed shipping, etc.). This

documentation will be provided to the laboratory supervisor to be forwarded to the DEO of the State of origin of the specimen. The laboratory supervisor will determine if the specimens are untestable and should be disposed.

**Collections that were not tested and have been disposed of due to extremely poor specimen quality will be reported immediately by the laboratory supervisor to the DEO and the ADD in the State from which the specimen originated.**

## **7.6 Preparation**

### **7.6.1 Preparation of the Specimen**

Before examinations begin, specimens must be prepared in order to ensure the quality of the serum and to allow for accuracy and validity when producing results. Specimens that have been stored in refrigeration shall be allowed to warm to room temperature before examination.

### **7.6.2 Centrifugation of the Specimen**

After specimens have been allowed to warm to room temperature, each collection or plant/slaughter facility kill date collection shall be centrifuged to separate serum from any clotting factors that remain in the specimen.

### **7.6.3 Grouping of Specimens**

Each collection shall be grouped by collection location or the plant/slaughtering facility and the kill/collection date. All corresponding information that is indicative of such collection shall be recorded on a worksheet that is produced within the laboratory. The information recorded on the worksheet at this step of preparation includes: collection location or plant/slaughtering facility name, establishment number, kill/collection date, received date, and State from which collection originated. All forms will accompany the collection from this step of preparation forward.

### **7.6.4 Disposal**

All caps/stoppers removed from each collection tube shall be retained or disposed of in preparation for examinations as per individual laboratory procedure.

## **7.7 Screening Examination**

### **7.7.1 Rapid Automated Presumptive (RAP) Test**

Collections that have been assessed and determined to be of good quality (collections containing adequate amounts of serum per specimen and displaying few to no signs of hemolysis, etc.) shall undergo the RAP test as outlined by NVSL protocol SOP-SERO-0021, as the screening examination. RAP test results shall be recorded as per [6.1 Screening and Secondary Examination Results](#) on VS Form 4-54 or VS Form 4-33.

Any specimen demonstrating an invalid RAP reading will be tested using the BAPA test. If the specimen produces an invalid result after performing the BAPA, it shall be

subjected to a FPA secondary examination. The results of the tests and information pertaining to the quality of the specimen shall be recorded and reported as per [6.1 Screening and Secondary Examination Results](#), [6.2.1 Specimen Quality Abbreviations](#), and [6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results](#).

#### **7.7.2 Buffered Acidified Plate Antigen (BAPA) Test**

Collections that have been assessed and determined to be of insufficient quality for the RAP examination shall undergo the BAPA test as outlined by NVSL protocol SOP-SERO-001, as the screening examination. BAPA test results shall be recorded as per [6.1 Screening and Secondary Examination Results](#).

Any specimen demonstrating an invalid BAPA reading will be tested using the FPA test. If the specimen produces an invalid FPA result, information pertaining to the quality of the specimen shall be recorded ([6.2 Reporting of Test Specimen Quality](#)) and reported ([6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results](#)).

#### **7.7.3 Positive Reactions**

Specimens that have produced positive reactions after having undergone a screening examination, either RAP or BAPA, shall undergo a FPA secondary examination.

#### **7.7.4 Invalid Results**

Specimens that have produced invalid results for the RAP or the BAPA test shall undergo a FPA secondary examination to attempt to obtain at least one result for a screening examination, unless otherwise specified by the laboratory supervisor in collaboration with the DEO of the State of origin of the specimen.

#### **7.7.5 Invalid Specimen**

Specimens determined to be invalid after having undergone a RAP or a BAPA examination will be noted as such on VS Form 4-54 or VS Form 4-33 using [6.2 Reporting of Test Specimen Quality](#).

### **7.8 Secondary Examination**

Any specimen yielding a non-negative test interpretation on a secondary examination shall remain at the laboratory and be preserved within the laboratory's archives. Serum from non-negative specimens, as determined by the secondary examination, shall be stored in a laboratory freezer, (-20 degrees C) and archived for no less than one year.

#### **7.8.1 Fluorescent Polarization Assay (FPA)**

All specimens that yield a positive RAP or BAPA test result shall be tested using the FPA test as outlined by NVSL protocols SOP-SERO-0030 (microplate test) and SOP-SEROp0031 (tube test). Results for all specimens that have undergone a FPA examination shall be recorded as per [6.1 Screening and Secondary Examination Results](#) on the VS Form 4-54, VS Form 4-33 and reported ([6.3 Protocol for Reporting Bovine Brucellosis Surveillance Specimen Test Results](#)).

### **7.8.2 Non-negative FPA Specimens**

All specimens that yield a non-negative test interpretation after having undergone a FPA test shall be retrieved and archived in the laboratory freezer at -20°C.

If a specimen yields a FPA non-negative test interpretation, the 9 specimens before and the 9 specimens after the non-negative specimen, along with the specimens' animal IDs, shall be retained by the laboratory until the laboratory supervisor, in collaboration with the DEO of the State of origin of the specimen, and a brucellosis CHVMO determine the specimens may be properly disposed of using laboratory procedures.

If a specimen yields a FPA non-negative test result, the specimen shall be subjected to a CF secondary examination, if the CF test is available. If the CF test is not available at the initial laboratory, the specimen should be submitted to NVSL. The DEO of the State of origin of the specimen shall provide a FedEx or UPS account number to cover charges for directing such requests to NVSL.

**If a specimen yields a non-negative FPA test interpretation, the specimen shall be forwarded to NVSL for confirmation of screening and secondary test interpretations.**

All non-negative secondary examination specimens shall be recorded as per [7.11 Recording ID for Non-negative Specimens on a new VS Form 4-54 or VS Form 4-33](#).

## **7.9 Additional Secondary Examinations**

### **7.9.1 Complement-Fixation Test (CF)**

Bovine brucellosis surveillance specimens that have undergone a screening examination followed by a FPA secondary examination and have produced a non-negative FPA test result shall then have a CF test performed as an additional secondary examination, as outlined by NVSL protocol SOP-SERO-0015.

Results for all specimens that have undergone a CF examination shall be recorded as per [6.1 Screening and Secondary Examination Results](#) on the VS Form 4-54, VS Form 4-33, or other VS approved forms.

### **7.9.2 Requests for additional secondary and supplemental tests**

Additional secondary tests and supplemental examinations may be requested by the DEO of the State of origin of the animal in consultation with a CHVMO. The DEO of the State of origin of the animal shall provide a FedEx or UPS account number to cover charges for directing such requests to NVSL if the tests are not available at the initial laboratory.

## **7.10 Retrieval of ID for Non-negative Specimens**

Bovine brucellosis surveillance serological specimens and specimens collected from deceased animals for all testing purposes that have yielded a non-negative test results on a screening or secondary examination shall have **all** physical identification, including but not limited to: any man-made identification devices, retrieved from the submitted collection for

that specimen. If possible, a photograph or colored, scanned image of any physical identification that has accompanied the specimen shall be created and filed with copies of all records within the laboratory. All physical identification of any non-negative specimens shall be recorded on or attached to the original VS Form 4-54 or VS Form 4-33 and be directed by the DEO of the State of origin of the specimen to the DEO for the State origin of the animal. **Do not clean any ID** as blood, hair or other tissues that are present on the ID may be used for DNA testing to correlate the ID to the blood specimen. The ID will be retained until the DEO of the State of origin of the animal determines, in consultation with a CHVMO, that retention of the ID is no longer necessary.

**In addition**, all IDs that accompanied the 9 specimens prior to the non-negative specimen and the 9 specimens following the non-negative specimen shall be retrieved by the AIC and retained until the DEO of the State of origin of the animal determines, in consultation with a CHVMO, that retention of the ID is no longer necessary.

If the ID did not accompany the blood specimen to the laboratory, the AIC of the State of origin of the specimen shall retrieve the IDs and send them to the DEO of the State of origin of the animal. The IDs shall be retained by of the State of origin of the animal until it's determined, in consultation with a CHVMO, that retention of the ID is no longer necessary.

## ***7.11 Recording ID for Non-negative Specimens on a New VS Form 4-54 or VS Form 4-33***

### **7.11.1 Recording of ID for Non-Negative Specimens**

Screening examination non-negative specimens shall have all ID ([2.3 Identification Required](#)) retrieved and recorded on a new, separate VS Form 4-54 or VS Form 4-33 and all test results shall be recorded on the new VS Form 4-54, VS Form 4-33 in the appropriate column for each examination. A copy of the new VS Form 4-54 or VS Form 4-33 and VS Form 10-4 shall accompany the original forms and be sent (initially electronically followed by hard copy mailed), to the DEO of the State of origin of the specimen. If the IDs did not accompany the specimens to the laboratory, as per the collection fee-basis agreement, the AIC shall retrieve and retain the IDs until the DEO, in collaboration with a CHVMO, determines that retention of the IDs is no longer necessary. If the screening test non-negative specimen is referred to NVSL, a copy of the new VS Form 4-54 or VS Form 4-33 and VS Form 10-4 shall accompany the specimen to NVSL.

### **7.11.2 Recording of ID for Specimens Prior To and Following Non-Negative Specimens**

All IDs from the 9 specimens prior to and the 9 specimens following the non-negative specimen in that particular collection shall be retrieved and recorded, along with all examination results, on the new VS Form 4-54 or VS Form 4-33. The retrieved ID from these specimens shall be retained at the laboratory or directed to the DEO or AIC for the State of origin of the specimen. If the IDs did not accompany the specimens to the laboratory, the AIC shall retrieve and retain the IDs. The IDs will be retained until the DEO, in collaboration with a CHVMO, determines that retention of the IDs is no longer necessary.

### ***7.12 Retention of Records***

A copy of all original forms shall be retained or re-produced and stored in the laboratory. This includes: original VS Form 4-54, original VS Form 4-33, VS-Form 10-4, and the new VS Form 4-54, photographs and scanned images of all ID correlated with non-negative specimens, any kill/log/lot sheets, and laboratory work sheets. All records related to non-negative specimens shall be archived within the laboratory for 10 years.